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ORIGINAL ARTICLE

Evaluation of the Cepheid GeneXpert® system for detecting *Bacillus anthracis*

M.P. Ulrich[1], D.R. Christensen[1], S.R. Coyne[1], P.D. Craw[1], E.A. Henchal[1], S.H. Sakai[2], D. Swenson[2], J. Tholath[2], J. Tsai[2], A.F. Weir[2] and D.A. Norwood[1]

1 Diagnostic Systems Division, United States Army Medical Research Institute of Infectious Diseases, Frederick, MD, USA
2 Cepheid, 904 Caribbean Drive, Sunnyvale, CA, USA

Keywords
anthrax, automated system, *Bacillus anthracis*, GeneXpert®, nucleic acid, real-time PCR, sample processing.

Correspondence
D.A. Norwood, Diagnostic Systems Division, United States Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Frederick, MD 21702-5011, USA.
E-mail: david.norwood@amedd.army.mil

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Abstract

Aims: The Cepheid GeneXpert® is a four-site, automated sample preparation and real-time PCR detection system. In this study, the capability of the GeneXpert® to isolate and detect nucleic acid from *Bacillus anthracis* Ames spores was assessed.

Methods and Results: A four-plex, dried-down bead cartridge containing PCR reagents specific for the pXO1 and pXO2 plasmids as well as sample processing and inhibition controls was evaluated. For *B. anthracis* Ames spores harbouring pXO1 and pXO2, samples containing 68 CFU per ml (148 spores per ml) were positive in all four replicates. A limited cross-reactivity panel, which included closely related *Bacillus* species, was also tested to determine the specificity of the pXO1 and pXO2 assays. No cross-reactivity occurred. Further, *B. anthracis* Sterne spore samples were analysed to compare results when processed using the GeneXpert® to those run directly on the Cepheid SmartCycler® without sample processing. The GeneXpert® detection capability was three logs lower than the SmartCycler® indicating the benefit of incorporating a nucleic acid extraction procedure.

Conclusions: This study demonstrates that the GeneXpert® is a rapid and reliable system for simultaneously detecting the *B. anthracis* virulence plasmids pXO1 and pXO2.

Significance and Impact of the Study: The GeneXpert® is the only platform currently available that is capable of both nucleic acid purification and real-time PCR detection enclosed within a single system. Further, all sample manipulations are automated, thus reducing errors associated with manual processing.

Introduction

Advances in molecular biology have led to the use of PCR as a sensitive and specific method to detect and monitor micro-organisms in environmental samples. Successful detection of difficult-to-lyse organisms, including spores, requires efficient extraction of nucleic acid from the specimen as well as the removal of PCR inhibitors that may be present (Wilson 1997; de Kok *et al.* 1998; Kuske *et al.* 1998; Smith *et al.* 2003; Coyne *et al.* 2004). Real-time PCR provides an additional benefit, in which post-PCR manipulations, such as gel electrophoresis, are not required (Belgrader *et al.* 1999).

Traditionally, manual nucleic acid extraction methods have been used to obtain purified DNA or RNA for PCR analysis. With regard to those protocols, numerous disadvantages arise, including labour-intensive procedures, the necessity for specially trained staff, technician-dependent variability in the efficiency of extraction and cross-contamination because of manipulations required during processing (Kessler *et al.* 2001; Fiebelkorn *et al.* 2002; Germer *et al.* 2003; Knepp *et al.* 2003). In addition,

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14. ABSTRACT

Abstract Aims: The Cepheid GeneXpert(R) is a four-site, automated sample preparation and real-time PCR detection system. In this study, the capability of the GeneXpert(R) to isolate and detect nucleic acid from Bacillus anthracis Ames spores was assessed. Methods and Results: A four-plex, dried-down bead cartridge containing PCR reagents specific for the pXO1 and pXO2 plasmids as well as sample processing and inhibition controls was evaluated. For B. anthracis Ames spores harbouring pXO1 and pXO2, samples containing 68 CFU per ml (148 spores per ml) were positive in all four replicates. A limited cross-reactivity panel, which included closely related Bacillus species, was also tested to determine the specificity of the pXO1 and pXO2 assays. No cross-reactivity occurred. Further, B. anthracis Sterne spore samples were analysed to compare results when processed using the GeneXpert(R) to those run directly on the Cepheid SmartCycler(R) without sample processing. The GeneXpert(R) detection capability was three logs lower than the SmartCycler(R) indicating the benefit of incorporating a nucleic acid extraction procedure. Conclusions: This study demonstrates that the GeneXpert(R) is a rapid and reliable system for simultaneously detecting the B. anthracis virulence plasmids pXO1 and pXO2. Significance and Impact of the Study: The GeneXpert(R) is the only platform currently available that is capable of both nucleic acid purification and real-time PCR detection enclosed within a single system. Further, all sample manipulations are automated, thus reducing errors associated with manual processing.plasmids pX01 and pX02.

15. SUBJECT TERMS
methods, PCR, Cepheid GeneXpert, automation, Bacillus anthracis, detection, pX01, pX02

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discrepancies in real-time PCR (false positives *vs* false negatives) are often caused by extraction procedures (Loeffler *et al.* 2000; Kessler *et al.* 2001). Consequently, using an automated system capable of both nucleic acid preparation as well as real-time PCR detection should reduce manual input and the risk of human error.

Requirements for an adequate automated system include high sensitivity, versatility with sample matrices (i.e. environmental samples, swabs, whole blood), minimal technical knowledge, low maintenance and containment of pathogenic micro-organisms to reduce the risk of exposure (Petersen and McMillan 2002). In addition, physical disruption of the organism, especially difficult for gram-positive bacterial spores, is preferred because many lysis reagents inhibit PCR, resulting in the need for additional wash steps (Belgrader *et al.* 2000). For optimal results, an entirely automated system would include a mechanical disruption method and the ability to transfer fluids required for each PCR preprocessing step without human intervention (Belgrader *et al.* 2000).

The Cepheid GeneXpert® is a four-site, self-contained device integrating automated sample processing and real-time PCR detection of infectious agents. All steps required for identifying bacterial and viral threat agents in various biological specimens, including sample preparation, amplification and detection, are combined within a single instrument that provides results in approx. 30–40 min. The time required for sample processing is approx. 5 min with the remaining time used for real-time PCR detection. The GeneXpert® is designed to utilize self-contained sample-preparation cartridges that eliminate complex manual procedures as well as amplicon or agent contamination and PCR inhibitors (Petersen and McMillan 2002). Cartridges are preloaded with lyophilized PCR reagents in bead form that target specific organism(s) and are designed for single-use purposes. In addition, each of the four sites is operated and controlled independently, allowing for the use of four different sample-processing and thermal-cycling protocols.

The GeneXpert® extraction procedure incorporates a membrane where cells are separated from the sample matrix, concentrated and washed to remove inhibitors. Sonication, integrating ultrasonic energy and glass beads, mechanically lyses the retained cells. The purified nucleic acid is then combined with PCR amplification reagents and delivered to the reaction chamber of the cycling tube (Petersen and McMillan 2002). Each site contains an I-CORE (intelligent cooling/heating optical reaction) module for thermal cycling and real-time detection of PCR amplicons (GeneXpert® Operator manual, Cepheid, Sunnyvale, CA, USA). The I-CORE optical system allows for excitation and detection of four different spectral bands, so up to four targets can be detected simultaneously in a single reaction tube (Petersen and McMillan 2002).

The focus of this investigation was to determine the limit of detection (LOD) of the GeneXpert® system for *Bacillus anthracis* Ames spores spiked into aqueous air collection samples taken from various sites that are known to be negative for the presence of *B. anthracis*. The lyophilized PCR reagents used for this study were designed to identify the *B. anthracis* pXO1 and pXO2 plasmids, which encode protective antigen and capsule genes, respectively. With the incorporation of internal controls for PCR inhibition (IPC) and sample processing (SPC), the findings of this investigation were designed to demonstrate the capability of the GeneXpert® automated platform to provide a rapid and reliable method for detecting *B. anthracis*.

Materials and methods

Bacillus preparation

Bacillus strains were obtained from culture collections maintained at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID, Frederick, MD, USA). Before sample processing, *B. anthracis* Ames and Sterne spores were washed to remove extraneous DNA using a proprietary method developed by Cepheid. Washed spores from each stock suspension were enumerated by plating tenfold dilutions in duplicate on sheep blood agar (SBA) (Remel, Lenexa, KS, USA) and incubated at 35°C for 24 h.

Comparison of CFU enumeration and spore counts

The relationship between colony forming units (CFU) and spore counts was determined from the stock of *B. anthracis* Ames washed spores used in this study. Spore counts were determined using a Petroff–Hauser chamber and CFU titres were established by plating on SBA as previously described.

Limit of detection for *Bacillus anthracis* Ames spores using the GeneXpert®

Liquid eluates from multiple air sampling systems were combined to provide sufficient volume for the dilutions performed in this study as well as to normalize the effects of potential PCR inhibitors that may be present in the eluates across all tested samples. Aliquots (1 ml) of *B. anthracis* Ames spores were diluted in combined air collection eluates (9 ml) with concentrations ranging from $8{\cdot}3 \times 10^4$ to $1{\cdot}27 \times 10^1$ CFU per ml. The diluted spores were counted by plating on SBA as described

previously to determine CFU per millilitre of extracted sample. A volume of 1 ml of each dilution was added to the cartridges as sample, and four replicates of each concentration were analysed. *Bacillus anthracis* Ames samples were tested using multiplexed assays for pXO1 (accession number: M22589·1), pXO2 (accession number: M24150), IPC (Hartman *et al.* 2004) and SPC. All GeneXpert® protocols were provided by Cepheid.

Cross-reactivity

Seven additional stocks of *Bacillus* (*B. anthracis* Pasteur, *B. anthracis* Sterne, *B. anthracis* Vollum, *B. cereus* mycoides NRS 936, *B. subtilis* Niger, *B. thuringiensis* Los Alamos, *B. thuringiensis* NCTC 4041) were prepared and washed to remove extraneous DNA utilizing the Cepheid protocol. Stock suspensions of washed spores were enumerated by plating tenfold dilutions in duplicate on SBA as previously described. High (1×10^5 to 1×10^4 CFU per ml) and low (1×10^3 to 1×10^2 CFU per ml) concentrations of each spore stock were made in water. Test samples were prepared by spiking 900 μl of clean air sampler buffer (phosphate-buffered saline and triton X-100) with 100 μl of the high and low dilutions. The bacterial spore preparations were randomized with ten negative control samples and tested using the four-plex cartridges as described above.

Limit of detection for *Bacillus anthracis* Sterne spores

Tenfold serial dilutions of washed *B. anthracis* Sterne spores, beginning with approx. 1×10^6 CFU per ml, were prepared in molecular biology grade water (Eppendorf, Westbury, NY, USA). In triplicate, 10 μl of each dilution was spiked into 510 μl of clean air sampler buffer and the entire volume was processed using the GeneXpert® with a cartridge targeting only the pXO1 plasmid. Comparison reactions were performed by spiking 10 μl of each dilution described above into 510 μl of clean air sampler buffer. From each dilution, 10 μl was analysed on the SmartCycler® in a 25-μl PCR reaction in duplicate without further processing.

Results

Comparison of CFU enumeration and spore counts

To ensure an accurate LOD, *B. anthracis* Ames spores were enumerated throughout the study by plating on SBA (CFU count). Initial characterization of the spore stock included plate counts in addition to determination of spore numbers using a Petroff–Hauser counting chamber. *Bacillus anthracis* Ames CFU counts were 46·0% of the actual spore numbers obtained using the Petroff–Hauser chamber. In the stock used for this investigation, 80% of the spores were refractive and a small number of clumps were observed, which is common in spore preparations. Because of these factors, the reduced CFU determinations were consistent with the condition of the spore stock. Final *B. anthracis* Ames spore calculations were obtained by multiplying the CFU count by a factor of 2·17 based on the ratio of spore numbers to CFU.

Limit of detection for *Bacillus anthracis* Ames spores using the GeneXpert®

The LOD for the pXO1 and pXO2 assays was 68 CFU per ml, which converts to 148 spores per ml (Figs 1 and 2). At the next lower dilution (49 spores per ml), two of

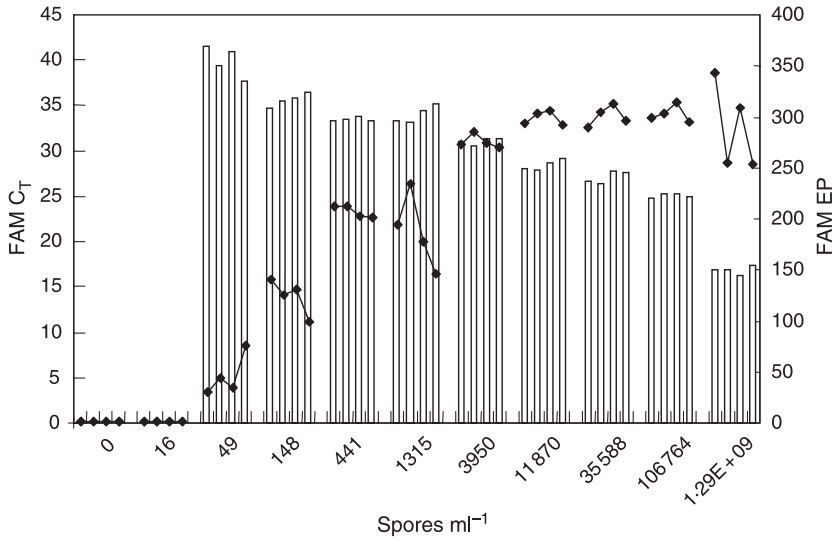

Figure 1 *Bacillus anthracis* Ames spores limit of detection for pXO1 using four-plex cartridges. Cycle threshold (FAM C_T) values are represented by bars and endpoint fluorescence (FAM EP) values are depicted using lines.

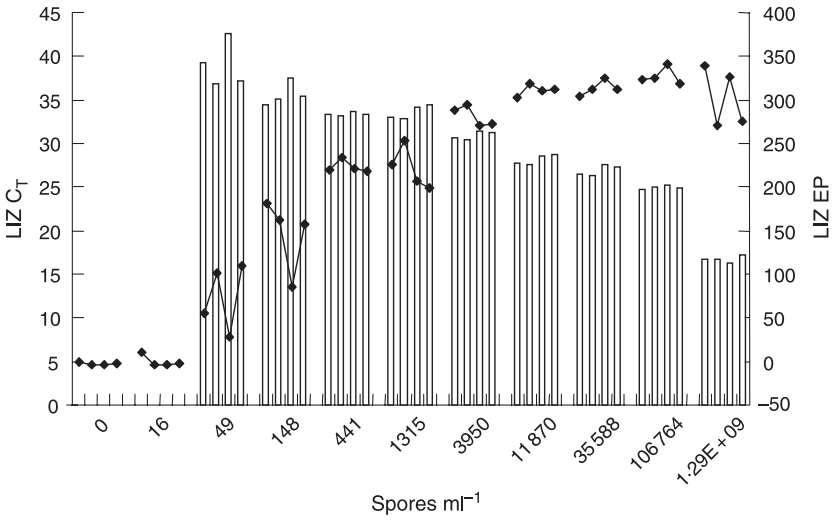

Figure 2 *Bacillus anthracis* Ames spores limit of detection for pXO2 using four-plex cartridges. Cycle threshold (LIZ C_T) values are represented by bars and endpoint fluorescence (LIZ EP) values are depicted using lines.

the four tests were positive (reached an adequate endpoint fluorescence threshold as determined by the software) for pXO1 and pXO2 (Figs 1 and 2). In addition, SPC results provided positive curves indicating that the sample processing method was working efficiently. When a spore concentration of ≥11 870 spores per ml was reached, competition for reagents occurred as a result of the high concentration of the sample, and SPC results were negative (data not shown). Further, inhibition controls indicated that there were no PCR inhibitors present based on the production of positive curves. Again, at a concentration of ≥11 870 spores per ml the sample caused a competition for reagents, and curves were negative (data not shown).

Cross-reactivity

There was no evidence of cross-reactivity with similarly related bacterial species (Table 1). In addition, the ten negative sample controls were not detected, indicating that no system cross-contamination occurred (Table 1).

Table 1 Cross-reactivity of *Bacillus* spores

Bacterial strain	Colony forming units per millilitre	pXO1	pXO2
B. anthracis Ames	$5{\cdot}2 \times 10^4$	POS	POS
	$5{\cdot}2 \times 10^2$	POS	POS
B. anthracis Pasteur	$8{\cdot}6 \times 10^4$	NEG	POS
	$8{\cdot}6 \times 10^2$	NEG	POS
B. anthracis Sterne	$2{\cdot}8 \times 10^4$	POS	NEG
	$2{\cdot}8 \times 10^2$	POS	NEG
B. anthracis Vollum	$9{\cdot}4 \times 10^4$	POS	POS
	$9{\cdot}4 \times 10^2$	POS	POS
B. cereus mycoides NRS 936	$4{\cdot}8 \times 10^4$	NEG	NEG
	$4{\cdot}8 \times 10^2$	NEG	NEG
B. subtilis Niger	$1{\cdot}1 \times 10^5$	NEG	NEG
	$1{\cdot}1 \times 10^3$	NEG	NEG
B. thuringiensis Los Alamos	$2{\cdot}5 \times 10^4$	NEG	NEG
	$2{\cdot}5 \times 10^2$	NEG	NEG
B. thuringiensis NCTC 4041	$4{\cdot}0 \times 10^4$	NEG	NEG
	$4{\cdot}0 \times 10^2$	NEG	NEG
Negative sample controls	0	NEG	NEG

Limit of detection for *Bacillus anthracis* Sterne spores

The LOD for the pXO1 assay determined using the GeneXpert® was approx. 1×10^3 CFU per ml (three positive replicates). At a dilution of approx. 1×10^2 CFU per ml, one of the three replicates was positive. For the dilutions tested using the SmartCycler® without sample processing, an LOD of 1×10^6 CFU per ml was achieved.

Discussion

Although recent advances in rapid and sensitive PCR procedures have been developed, there is a lack of fully automated methods for extracting and detecting micro-organisms. Automated instruments for sample processing and real-time PCR have numerous advantages when compared with manual methodologies. A decrease in preparation time is obtained, manual labour is reduced and human error, such as pipetting discrepancies and sample exchange, is eliminated (Smit *et al.* 2000). Integrated platforms must include an effective method for sample processing, incorporating an efficient lysis mechanism to purify nucleic acid as well as steps to remove inhibitors, in addition to containing stable PCR reagents that can accommodate the direct transfer of nucleic acid into the reaction (Petersen and McMillan 2002; Bailey *et al.* 2003).

The GeneXpert® contains glass beads within the cartridge that mechanically lyse organisms, including spores, when subjected to ultrasonic power. Previous studies

identified sonication as an effective method to rapidly disrupt bacterial spores for real-time PCR assays (Belgrader *et al.* 1999). Sonication improved the LOD by a decrease of three logs, reduced detection time and increased signal amplitude (Belgrader *et al.* 1999). The results of this investigation support those findings. The LOD for *B. anthracis* Sterne spores was decreased by three logs when comparing the GeneXpert® (1×10^3 CFU per ml) results to those of the SmartCycler® (1×10^6 CFU per ml). The LOD for Sterne spores using the GeneXpert® is increased over that for Ames spores because of the fact that the Sterne spores were tested using an earlier cartridge that was later improved to the four-plex design. However, the benefit of incorporating sample processing into the overall detection method was clearly depicted and it was not necessary to reproduce the trend with the four-plex cartridge. Petroff–Hauser cell counts were not performed for the Sterne study comparing the GeneXpert® to the SmartCycler®. Identical dilutions were used for each system allowing for direct assessment between samples. Exact spore counts were not necessary, as the evaluation of these two platforms was designed to show the benefit of using an integrated system capable of sample preparation and real-time PCR analysis over direct PCR analysis of a raw sample.

A concern with implementing automated systems that extract nucleic acid is the potential for cross-contamination of negative samples as a result of aerosolization or robotic error (Bailey *et al.* 2003; Knepp *et al.* 2003). All liquid handling by the GeneXpert®, for both sample preparation and amplification, occurs within the disposable cartridge, which prevents fluid cross-contamination. The closed system also reduces the risk of operator exposure to pathogenic organisms with the added benefit of a small overall instrument size that can be used within a biosafety cabinet. Results from this study show that the GeneXpert® can successfully extract and detect DNA from *Bacillus* spores without any cross-contamination. The ten negative samples included in the cross-reactivity study were randomly distributed among a set of *Bacillus* spore dilutions and there were no false positive results. In addition, the PCR reactions for detecting the virulence plasmids revealed curves for pXO1 in *B. anthracis* Ames, *B. anthracis* Sterne and *B. anthracis* Vollum, and for pXO2 in *B. anthracis* Ames, *B. anthracis* Pasteur and *B. anthracis* Vollum, as expected. All other *Bacillus* species produced negative results, further demonstrating the lack of sample cross-contamination. The organisms chosen to be included in the near neighbour panel were a small subset of 91 *Bacillus* species tested using the pXO1 and pXO2 assays incorporated into the GeneXpert® cartridges evaluated in this study (Christensen *et al.* 2006).

Besides reduction of specimen contamination, the extraction efficiency must be maintained for an automated platform to be successful (Exner and Lewinski 2003). In this study, the LOD for the pXO1 and pXO2 assays for *B. anthracis* Ames spores was 148 spores per ml (68 CFU per ml). Each of the four replicates was reproducible providing similar cycle threshold (C_T) and endpoint fluorescence values. Furthermore, the LOD obtained with the GeneXpert® for *B. anthracis* Ames spores was consistent with the LOD previously observed for both *B. anthracis* Ames and *B. anthracis* Sterne spores, which were tested in separate cartridges targeting either pXO1 or pXO2 (data not shown).

An additional benefit of the GeneXpert® is the capability for simultaneous detection of up to four targets in real time. The detection block was designed with four photodetectors containing several filters to capture signal data in separate spectral bands (Petersen and McMillan 2002). The cartridges used in this study were designed to detect pXO1 and pXO2 targets, an IPC to monitor PCR inhibitors that may cause false negative reactions (Hartman *et al.* 2004) and an SPC to verify whether sample processing within the cartridge worked properly. The SPC portion of the cartridge consists of a bead containing a micro-organism requiring sonication for cellular lysis. Within the cartridge, the SPC is mixed into and processed along with the experimental sample to be detected by real-time PCR. In this study, all controls (IPC and SPC) produced positive curves with consistent C_T values up to a sample spore concentration of ≥11 870 spores per ml. At this point, competition for reagents occurred as a result of the high concentration of the sample, and the curves for the two controls were negative. This result is due to a specific design and optimization of the controls to prevent competition with the agent-specific target assays. Therefore, high concentrations of *B. anthracis* spores produced negative SPC and IPC results. The failure of these controls is only critical when the target assays (pXO1 and pXO2) are negative. When negative results were found for pXO1 and pXO2, the SPC and IPC controls produced positive curves indicating the fact that the instrument was functioning optimally.

Several factors are important for PCR detection of specific target micro-organisms and include the availability of DNA from difficult-to-lyse cells (gram-positive *vs* gram-negative); the purity of the DNA from contaminants, which the GeneXpert® design addresses by incorporating a filter to concentrate cells and wash away inhibitors; and the condition of the extracted nucleic acid (Kuske *et al.* 1998). In addition, an automated system should provide rapid, efficient and reproducible results (Exner and Lewinski 2003). A platform such as the GeneXpert® requires minimal user input which reduces the probability of human processing errors, shortens the procedure time with

integration of nucleic acid extraction and real-time PCR and lessens the potential for cross-contamination. Further, the GeneXpert® is undergoing advanced development and testing for cartridges suitable to process more complex matrices such as whole blood. In a recent study, Raja *et al.* (2005) demonstrated the capability of the GeneXpert® to isolate RNA and analyse gene targets for lymph nodes from melanoma, breast cancer and lung cancer patients as well as analysis of melanoma metastatic to the lung, primary lung adenocarcinoma and healthy lung tissue. The expanding capability of the GeneXpert® is demonstrated by the RNA work performed in that study as well as the DNA testing described in this manuscript. Further development will only improve the potential uses for this instrument, especially considering the fact that there are no other platforms available that incorporate both sample processing and real-time PCR detection in one system.

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References

Bailey, A.M., Pajak, L., Fruchey, I.S., Cowan, C.A. and Emanuel, P.A. (2003) Robotic nucleic acid isolation using a magnetic bead resin and an automated liquid handler for biological agent simulants. *J Assoc Lab Automation* **8**, 113–120.

Belgrader, P., Benett, W., Hadley, D., Richards, J., Stratton, P., Mariella, R. and Milanovich, F. (1999) PCR detection of bacteria in seven minutes. *Science* **284**, 449–450.

Belgrader, P., Okuzumi, M., Pourahmadi, F., Borkholder, D.A. and Northrup, M.A. (2000) A microfluidic cartridge to prepare spores for PCR analysis. *Biosens Bioelectron* **14**, 849–852.

Christensen, D.R., Hartman, L.J., Loveless, B.M., Frye, M.S., Shipley, M.A., Bridge, D.L., House, M.J., Richards, M.J., Kaplan, R.S., Garrison, J., Baldwin, C.D., Kulesh, D.A. and Norwood, D.A. (2006) Detection of biological threat agents by real-time PCR: Comparison of assay performance on the R.A.P.I.D., the LightCycler, and the SmartCycler platforms. *Clin Chem* **52**, 141–145.

Coyne, S.R., Craw, P.D., Norwood, D.A. and Ulrich, M.P. (2004) Comparative analysis of Schleicher and Schuell IsoCode Stix DNA isolation device and the Qiagen QIAamp DNA mini kit. *J Clin Microbiol* **42**, 4859–4862.

Exner, M.M. and Lewinski, M.A. (2003) Isolation and detection of *Borrelia burgdorferi* DNA from cerebral spinal fluid, synovial fluid, blood, urine, and ticks using the Roche MagNA Pure system and real-time PCR. *Diagn Microbiol Infect Dis* **46**, 235–240.

Fiebelkorn, K.R., Lee, B.G., Hill, C.E., Caliendo, A.M. and Nolte, F.S. (2002) Clinical evaluation of an automated nucleic acid isolation system. *Clin Chem* **48**, 1613–1615.

Germer, J.J., Lins, M.M., Jensen, M.E., Harmsen, W.S., Ilstrup, D.M., Mitchell, P.S., Cockerill, F.R. and Patel, R. (2003) Evaluation of the MagNA Pure LC instrument for extraction of hepatitis C virus RNA for the COBAS AMPLICOR hepatitis C virus test, version 2.0. *J Clin Microbiol* **41**, 3503–3508.

Hartman, L.J., Coyne, S.R. and Norwood, D.A. (2004) Development of a novel internal positive control for Taqman based assays. *Mol Cell Probes* **19**, 51–59.

Kessler, H.H., Mühlbauer, G., Stelzl, E., Daghofer, E., Santner, B.I. and Marth, E. (2001) Fully automated nucleic acid extraction: MagNA Pure LC. *Clin Chem* **47**, 1124–1126.

Knepp, J.H., Geahr, M.A., Forman, M.S. and Valsamakis, A. (2003) Comparison of automated and manual nucleic acid extraction methods for detection of enterovirus RNA. *J Clin Microbiol* **41**, 3532–3536.

de Kok, J.B., Hendriks, J.C., van Solinge, W.W., Willems, H.L., Mensink, E.J. and Swinkels, D.W. (1998) Use of real-time quantitative PCR to compare DNA isolation methods. *Clin Chem* **44**, 2201–2204.

Kuske, C.R., Banton, K.L., Adorada, D.L., Stark, P.C., Hill, K.K. and Jackson, P.J. (1998) Small-scale DNA sample preparation method for field PCR detection of microbial cells and spores in soil. *Appl Environ Microbiol* **64**, 2463–2472.

Loeffler, J., Henke, N., Hebart, H., Schmidt, D., Hagmeyer, L., Schumacher, U. and Einsele, H. (2000) Quantification of fungal DNA by using fluorescence resonance energy transfer and the Light Cycler system. *J Clin Microbiol* **38**, 586–590.

Petersen, K. and McMillan, W. (2002) IVD systems in bioterrorism response. *IVD Technology* **May**, 35.

Raja, S., Ching, J., Xi, L., Hughes, S.J., Chang, R., Wong, W., McMillan, W., Gooding, W.E. *et al.* (2005) Technology for automated, rapid, and quantitative PCR or reverse transcription-PCR clinical testing. *Clin Chem* **51**, 882–890.

Smit, M.L., Giesendorf, B.A., Heil, S.G., Ver, J.A., Trijbels, F.J. and Blom, H.J. (2000) Automated extraction and amplification of DNA from whole blood using a robotic workstation and an integrated thermocycler. *Biotechnol Appl Biochem* **32**, 121–125.

Smith, K., Diggle, M.A. and Clarke, S.C. (2003) Comparison of commercial DNA extraction kits for extraction of bacterial genomic DNA from whole-blood samples. *J Clin Microbiol* **41**, 2440–2443.

Wilson, I.G. (1997) Inhibition and facilitation of nucleic acid amplification. *Appl Environ Microbiol* **63**, 3741–3751.